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(57) Abstract

Systems and methods are disclosed for removing redox-active substances from aqueous, partially aqueous or non-aqueous fluids. The invention involves contacting the fluid to be treated with a redox-active agent that is insoluble in the fluid, thereby oxidizing interfering redox-active substances. Electron transfer agents can also be employed. The residual redox oxidizing agent is removed from the treated fluid on the basis of its insolubility, so that no residual redox activity remains. The invention is useful for removal of interfering redox-active substances from liquid samples when analytes in the sample are to be measured using reduction-oxidation chemistry and the redox-active interfering substances removed by the disclosed method interfere in the reduction-oxidation analysis. Furthermore, this invention is especially useful to pretreat blood, serum, plasma or other bodily fluids prior to analysis or other use of these fluids, where the presence of reduction-oxidation active substances constitutes an interference in that analysis or use.

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THE USE OF FLUID INSOLUBLE OXIDIZING AGENTS TO ELIMINATE INTERFERING SUBSTANCES IN OXIDATION-REDUCTION MEASURING SYSTEMS

BACKGROUND OF THE INVENTION

The invention generally relates to more accurate redox measuring devices. Analytical devices based on redox reactions can provide inaccurate measurements due to the presence of redox-active contaminants.

This invention relates generally to a system for removing, eliminating or rendering non-interfering redox-active contaminants contained in fluids by the use of redox-active insoluble compounds (hereinafter, RAIC), methods for performing the removal and the resulting fluid free of the interfering redox-contaminants.

The invention also relates to diagnostic kits which contain the redox-active insoluble compound used in accordance with the invention. Improved assays are obtained as a result of the invention.

The invention relates to disposable, machine-independent devices and to machine or apparatus-dependent devices, not disposable after each use.

The fluid free of the interfering redox-active contaminants is then suitable for a variety of uses including a redox reaction based analysis of analytes in the fluid using redox measuring systems. The analysis is more accurate

than if performed without the use of the redox-active insoluble compound.

This system depends on oxidation-reduction (redox) chemical reactions for the removal of the unwanted or contaminating redox-active components. The behavior of the redox-active insoluble compounds and the interfering redox-active contaminants which are sought to be rendered non-interfering (or inert) in the analytical reaction for the analyte (as well as various analysis techniques) can be understood by reference to the general principles of oxidation-reduction reactions.

For the purposes of facilitating the understanding of the following discussion, description and examples set forth in this disclosure, the following definitions are given which are intended to supplement or complement the terms of art generally accepted.

Insoluble - a material is insoluble if a fluid that passes over (or contacts it) picks up an amount of said material that is wholly insignificant compared to the concentration of the analyte(s) of interest in said fluid. As a general matter, insoluble material will not dissolve to an extent in excess of 10 ppm, regardless of analyte concentration.

Redox reaction - a reaction in which electrons are liberated by one substance while simultaneously being consumed by another substance. The substance that loses the electrons is the reducing agent, or reductant, and is itself oxidized. The substance that gains electrons is the oxidizing agent, or oxidant, and is itself reduced. In redox reactions, neither oxidation nor reduction can occur alone. It can also be defined in terms of change in oxidation number - the oxidizing agent undergoes an algebraic decrease in oxidation number, and the reducing agent undergoes an algebraic increase. In redox measurement systems, redox reactions are typically catalyzed. Reactions catalyzed by proteins include enzymatic reactions; reactions can also be

non-protein catalyzed, as is the case in the basic reducing sugar assay.

Redox measurement system - a redox reaction, or a set of redox reactions that act on some redox-active analyte to give a product of reaction; the product then being converted to some signal that can be interpreted and used to detect the absence or presence and determine the concentration of the analyte. Examples of this measurement include colorimetric measurement, as in the case where the product of the reaction is a color change in a dye molecule, and electronic measurement, as in the case where the redox reaction is linked to a semiconductor on a biosensor.

Redox-active - compounds which can undergo oxidation or reduction in redox reactions under the conditions present in the fluid, i.e., reducing and oxidizing agents.

Analyte - a redox-active substance whose absence or presence or concentration is measured by a redox measurement system.

Fluid - any liquid substance, either aqueous, non-aqueous or any mixture of the two, that can be contacted with an insoluble redox-active material. The fluid may be subsequently analyzed in a redox measurement system, but does not have to be so analyzed.

Interfering - a substance, especially a redox-active substance, that interferes with a redox measurement system, usually by itself being oxidized or reduced, thereby generating an inaccurate result from the redox measurement system. Thus a redox based analysis of a fluid with an interfering substance would be less accurate than if the analysis were performed without an interfering substance. This term in the invention is interchangeable with "contaminating". For fluids to be used in ways other than analysis, "interfering" means causing error or non-ideal performance in the use of the product fluid.

The "interfering" substance will generally cause a lower reading of the concentration of the analyte, unless rendered "non-interfering" in accordance with the invention.

Render non-interfering - refers to the effect the insoluble redox-active compound has on the redox based analysis of an analyte containing fluid, which is to make such analysis more accurate. This is achieved by the interaction of the insoluble redox-active agent with redox-active contaminants in the fluid. As a result the contaminant is oxidized if the RAIC is an oxidizing agent or the contaminant reduced if the RAIC is a reducing agent. This interaction causes the contaminant or contaminants to be inert, inactive or to not interact with the compounds responsible for the redox based analytical determination. The presence of the RAIC in the system causes the analytical reaction to be as accurate as if there were no contaminant present. Although applicants do not intend to be bound by any theory on the effect of the redox-active insoluble compound, it is thought that the RAIC reacts with respect to the interfering contaminant by way of a redox reaction, which precedes the principal redox reaction which involves the analytical determination of the analyte. It would appear that there is a net positive electromotive potential between the RAIC and redox-active contaminant and a pathway exists for electron transfer between them while no effective pathway exists for electron transfer between the RAIC and analyte of interest. Thus the RAIC reacts selectively with the contaminants while not affecting the analyte. When oxidized, the reducing interfering compound is not interfering in the following redox reaction.

Oxidation - reduction (redox) reactions are very common and important chemical reactions. Many different types of compounds can undergo redox reactions. As is well known, a molecule is oxidized when it undergoes an algebraic increase in oxidation number, and a molecule is reduced when it undergoes an algebraic decrease in oxidation number.

Oxidation or reduction cannot occur without the other also occurring. Thus a substance cannot be reduced without another substance being oxidized. When a substance is reduced, it is responsible for the oxidation of another substance, so the substance that is reduced is called the oxidizing agent. Conversely, the substance that is oxidized is called the reducing agent, because it is responsible for the reduction of another substance.

For redox reactions, the reader is referred to the literature, for instance House, Modern Synthetic Reactions (W.A. Benjamin, Inc., Menlo Park, CA, 1972) discusses at length various types of redox reactions. In chapter 2, House describes metal halide reactions; chapter 3 describes dissolving metal reductions; chapter 4 describes reductions with hydrazine; chapter 5 describes oxidation with chromium and manganese compounds; chapter 6 describes oxidation with peracids and other peroxides; and chapter 7 describes other methods of oxidation. Mortimer, Chemistry: a Conceptual Approach (D. Van Nostrand Co., New York, 1979) gives an example of a typical redox reaction which occurs in acid solution, wherein MnO_4^{-1} is oxidized to Mn^{2+} while As_4O_6 is reduced to H_3AsO_4:

$$24\ H^+ + 18\ H_2O + As_4O_6 + 8\ MnO_4^- \longrightarrow 20\ H_3AsO_4 + 8\ Mn^{+2}$$

Also see Manahan, Environmental Chemistry (Willard Grant Press, Boston, 1984) which gives an example of a redox reaction in alkaline conditions, in which toxic, soluble chromium(VI) is removed from waste water by reducing it to insoluble chromium(III):

$$8\ CrO_4^{-2} + Na_2S + 20\ H_2O \longrightarrow$$
$$4\ Cr_2S_3 + 3\ SO_4^{2-} + 40\ OH^- + 30\ Na^+.$$

These references are incorporated herein by reference.

The occurrence or non-occurrence of a redox reaction between any two chemical compounds will depend upon two factors: (1) whether there is a driving force favoring the reaction, i.e., does the energy of the potential reactants exceed the energy of the potential products of a

redox interaction, and (2) whether there is a pathway for the electrons to transfer from reducing agent to oxidizing agent under the conditions prevailing in the system. As is well known in the art, if there exists a potential energy difference between reactants and products such that energy could be given up by the reaction rendering the products at a lower final energy than the reactants, and if a means exists for electrons to transfer from reducing agent to oxidizing agent under the conditions prevalent in the system, then the redox reaction will occur.

The second condition is important. It is well known that most organic compounds of intermediate or high reduction state are essentially stable in the presence of atmospheric oxygen, yet they could give up energy if a redox reaction occurred between them and oxygen. An aqueous solution containing ethyl alcohol and glucose, for instance, could oxidize to carbon dioxide and water by reaction with oxygen, as is well known by those skilled in the art. But an energy barrier exists for the transfer of electrons from these molecules to oxygen such that these potential redox reactions do not occur or occur at insignificant rates under conditions of neutral pH and temperatures of 0-40°C. Redox reaction between these materials and oxygen can be facilitated by a rise in ambient temperature that overcomes the energy barrier, or by the addition of catalysts to provide a pathway for electrons between interacting redox compounds.

In general organic compounds undergo redox reactions, although, in comparison to inorganic compounds, organic molecules more frequently require a catalyst to facilitate redox reactions. Enzymes are especially important catalysts of organic redox reactions under conditions restricted to the neutral pH range and temperatures from 0 to about 40°C. Enzymes catalyzing redox reactions fall into classes including, but not limited to, the dehydrogenases, the oxidases, the oxygenases and other oxidoreductases, as is well known in the art and described in Biochemistry, 2nd ed.,

A.L. Lehninger, Worth Publishers, Inc. N.Y. (1975) pp. 477-508.

Other than enzyme catalysts, there are other known catalysts that facilitate redox reactions of organic molecules with each other, or with inorganic or organometallic redox partners. These catalysts can generally be termed electron transfer agent(s) (etas) since they reversibly transfer electrons among the redox-active compounds being alternatively oxidized and reduced in a redox reaction. In these reactions, the etas are not consumed but recycle between the oxidized and reduced forms, facilitating the reaction. There are a great number of chemical substances that can play this catalytic role in redox chemistry, including but not limited to simple metal ions such as iron in its (II-III) valences, copper (I-II), Cr (II-III), among many other metal ions; and many organic compounds including, but not limited to phenazine methosulfate (PMS), 2,6-dichlorophenolindophenol (DCIP), 1,4-benzoquinone and many of its substituted forms, phenazine-, phenoxazine- and phenothiazine-based dyes including but not limited to methylene blue, azure blue A, basic blue 3, neutral red, among a very large number of other redox-active dyes, as is well known to those skilled in the art.

It is to be noted that reduced chemical compounds can be selectively oxidized in the presence of each other by addition of redox catalysts that selectively interact with one or the other reduced compound, for whatever reason. For example, at about pH 9 the addition of iron (III) ion to the aqueous solution of alcohol and glucose mentioned above will facilitate oxidation of the glucose, with concomitant reduction of oxygen, but will not similarly facilitate oxidation of the ethanol. Alternatively, addition of enzyme catalysts such as glucose oxidase will cause oxidation of glucose without oxidation of ethanol, and addition of alcohol oxidase will cause oxidation of the alcohol without oxidizing the glucose.

Selective oxidation of reduced analytes in mixtures of compounds by the addition of a redox catalyst that selectively interacts with an analyte of interest is a common and powerful means to determine the presence and to determine the concentration of analytes, as is well known to those skilled in the art. In general, the electrons provided by change in oxidation state of the analyte is converted to a measurable signal by a number of means, such as coupling the oxidation to a colorimetric system as was done in the test used to measure glucose, disclosed in U.S. Patent 4,391,906. Alternatively, a signal can be generated by coupling the oxidation to an electronic system, as is done with enzyme electrodes.

A problem arises in using redox chemistry to detect and determine analytes when there are redox-active substances present in the sample along with the analyte. The signals obtained in colorimetric and electronic systems typically result from the total of electrons available from redox-active substances in the system. Non analyte redox-active materials may be present in the sample that will give up their electrons to the colorimetric or electronic signal producing system, and thereby provide a falsely high signal compared to the concentration of analyte.

One common means to correct for such redox-active interfering materials is to measure the total redox signal of the system in two samples, one containing an analyte - selective redox catalyst and one without catalyst, determining the content of analyte by difference. Obviously, this approach requires at least twice as much effort, sample and analytical materials to determine the analyte concentration and is also inherently less accurate than a direct analysis.

Another common means to correct for the presence of the interfering contaminants is to destroy them prior to initiation of the analysis by addition of an excess of an oxidizing agent.

Rendering interfering reducing substances non-interfering by addition of oxidants is fraught with difficulty. First, the oxidant added must not oxidize the analyte of interest. Thus, it must be of an energy level able to spontaneously react with the interfering reducing substances and it must have a pathway for electrons from interfering materials to itself, but not from the analyte of interest. More importantly, addition of an oxidant typically leaves a residue of oxidant in the sample. This residue itself constitutes an interfering contaminant because it is capable of accepting electrons from the oxidation of the analyte in the analysis step, resulting in an incorrect, low signal relative to the analyte. Typically, as shown in the prior art, the oxidant added to remove interfering reducing substances must itself be removed, without any significant residue, by a back titration with a known reducing agent. Alternatively, the fluid treated by the oxidizing agent may be left with the residual agent in place, but the fluid is not subsequently used in a manner involving redox reactions. This way of correcting for the interfering materials is not satisfactory.

The generalized description of redox analysis of specific analytes in complex fluids, and interference with this analysis by contaminating reducing (or oxidizing) substances, is frequently encountered. For example, the colorimetric assay of many biologically important analytes is mediated by the contact with analyte-specific dehydrogenases and the resultant oxidation/reduction of nicotinamide adenine nucleotide (NAD) or its phosphate analogue NADP. In reduced form, NAD(P)H can be measured by reaction with tetrazolium salts to yield an equivalent amount of highly colored formazan dye. For instance, NADH plus iodophenyl nitrophenyl phenyltetrazolium (INT) in the presence of a diaphorase will yield NAD and INT-formazan, which is dark red. The amount of red color produced by this redox reaction is proportional to the concentration of NAD(P)H; however, if an interfering reducing agent were present in this sample, then a more

intense signal will be generated, thereby, causing an overestimation of the concentration of NADH. This technology is utilized by U.S. Patent Numbers 3,867,259; 4,024,021; 4,247,833; and 4,556,834, all of which are subject to interference by reducing substances as described.

Another redox measurement technology that determines metabolite or chemical levels in a fluid utilizes enzyme oxidases. Numerous examples of this technology exist in the patent literature, for example see U.S. Patent 4,391,906; U.S. Patent 3,164,534; and U.S. Patent 4,544,249 which deal with methods for eliminating bilirubin interference, and U.S. Patent 4,186,251. All of these patents involve the very well known assay system in which the compound to be measured is contacted with an oxidase in the presence of oxygen, resulting in hydrogen peroxide production. The resulting hydrogen peroxide is then utilized to oxidize a chromogen to produce an indicative dye. It is clear that the presence of redox-active components in these systems will lead to incorrect results, as is well known in the art. Indeed, the package insert accompanying the Boehringer Mannheim Reflotron Cholesterol diagnostic states "The following substances, when present in high or pathologic concentrations, may produce depressed cholesterol values: cysteine, ascorbic acid, methyldopa, gentisic acid, dipyrone, ampyrone, homogentisic acid, or glutathione". All of these listed compounds are interfering reducing agents, as will be readily apparent to those of average skill in the art. Young et al. have published a comprehensive list of redox-active compounds that may be present in blood that can lead to inaccuracies in redox-based measurements of blood analytes (Clin. Chem. 21, No. 5, 1975). The list of these interfering contaminants is incorporated herein by reference. The interference of all the reducing agents listed above referred to in Young et al., may be eliminated by the use of the present invention.

Still another technology that makes use of redox reactions is the enzyme electrode. For instance, the

oxidation of glucose will produce hydrogen peroxide, the rate of whose production can be measured by a current at the surface of the electrode. One such apparatus is disclosed in U.S. Patent No. 4,340,448, which discusses the potentiometric determination of hydrogen peroxide. Some other examples of this technology are U.S. Patent No. 4,547,280, which describes a maltose sensor, and U.S. Patent No. 4,356,074, which describes substrate-specific galactose oxidase electrodes. Since these electrodes are dependent on redox reactions, interfering substances that are redox-active may generate incorrect readings.

Newer measurement systems that use redox chemistry can come in the form of biosensors. This is a generic term that is not specific to any measurement type, but one embodiment of biosensors involves attaching a redox-active biological material to a semiconductor. The behavior of the semiconductor and, therefore, the signal generated by the semiconductor, is dependent upon the redox state of the biological material. In this field, as in the more traditional bio-measurement fields discussed above, it is important that the redox state of the particular measurement system, in this case, the redox-active biological material, be affected only by the analyte. Therefore, if an interfering redox-active substance is present in the fluid to be measured, an erroneous signal will be generated.

Pending patent application serial number 075,817, discloses a method for decreasing the color produced by colorimetric redox-measuring systems. The disclosure discusses the oxidation of NAD(P)H and the simultaneous reduction of a chromogen, the reaction being catalyzed by diaphorase. In that system, the concentration of NAD(P)H is such that too much color would be produced by the chromogen, so that the concentration of NAD(P)H cannot be determined. Therefore, an alternate electron acceptor is introduced that competes for electrons with the chromogen. The alternate electron acceptor is by definition an oxidizing agent. Therefore, problems may arise when a contaminating reducing

agent is present; the contaminating agent can reduce the alternate electron acceptor, thereby giving a false reading.

BRIEF DESCRIPTION OF THE PRIOR ART

Typical of the conventional technology which adds oxidizing or redox-active agents to biological fluids in which the addition of such agents are made for various reasons are the following issued patents.

U.S. Patent No. 4,342,740 describes the use of a soluble oxidizing agent which is added to facilitate the labeling of red blood cells with Technetium - 99 M. The resulting fluid contains residual oxidizing agent and the fluid is not subsequently analyzed by redox analysis.

U.S. Patent No. 4,180,592 describes a process for decolorizing blood by the addition of the soluble oxidizing agent hydrogen peroxide and the removal of excess peroxide. The end use of the decolorized blood product is not analyzed by redox methods.

U.S. Patent No. 4,298,688 describes a test strip to measure glucose in body fluids. The strip contains an oxidizing agent to destroy interferents. The residual oxidizing agent and glucose are separated by chromotography. The patent does not teach nor suggest the use of insoluble oxidizing agents and their removal based on insolubility.

U.S. Patent No. 4,255,385 describes a method, reagent and test kit for the determination of glycosylated hemoglobin with use of the soluble oxidizing agent, ferricyanide. The treated fluid contains residual oxidizing agent and is not analyzed by redox reactions.

U.S. Patent No. 3,894,844 describes a method for determining the amount of triglycerides, cholesterol and phospholipids present in blood by using oxidation reactions. The three analytes of interest are separated in a procedure which removes interfering reducing substances as well.

U.S. Patent No. 4,256,833 describes the preparation of a peroxidase-coupled IgG antibody by formation of a Schiff's base. The resulting product is precipitated and recovered from the reduction reaction mixture effectively

removing it from the residual reducing agent before subsequent use. Insoluble reducing agents are not taught nor suggested.

U.S. Patent No. 4,645,660 describes the addition of a soluble reducing agent to a radioactive diagnostic agent wherein iron ions compete with the desired radioactive elements for formation of desired chelate compounds, and a reducing agent is added during formation of these chelates to reduce the iron to a form that does not effectively chelate. In this patent the resulting fluid contains the residual reducing agents and the fluid is not subsequently analyzed by redox methods.

U.S. Patent No. 4,720,385 describes the sterilization of therapeutically or immunologically active protein solutions by contacting these solutions with a metal chelate complex such as copper phenanthroline and a reducing agent, such as ascorbic acid or a thiol. The resulting solution contains the soluble residual reducing agent and is not subsequently analyzed by redox methods.

Patents dealing with the addition of oxidizing agents to solutions containing various contaminants, for the purpose of removing the contaminants include U.S. Patent No. 4,572,797, describing the addition of soluble oxidizing agents to aqueous solutions containing trace metals. In this patent, the oxidized product of the trace contaminant is insoluble in the liquid is then separable from the fluid as a precipitate. The residual oxidizing agent remains in solution and the resulting fluid is not analyzed by redox methods.

Similarly, U.S. Patent No. 4,431,847 describes the addition of an oxidizing agent to fluids containing halogenated phenolic compounds, to cause the polymerization and insolubilization of these compounds. The resulting polymeric form of the contaminants are easily removed as precipitates. The resulting fluid contains the residual oxidizing agent and is not subsequently analyzed by redox methods.

U.S. Patent No. 4,249,939 describes the removal of copper from spent solutions of complexing agents containing cuprous aluminum tetrahalides. In this patent, the copper is oxidized to the water soluble cupric form by contacting the fluid with an oxidizing agent. Organic materials in the solution are removed by extraction with an organic solvent, and the copper is subsequently recovered by contacting the aqueous phase with a metal of higher electromotive potential than copper, plating the copper out of the solution. In this last step, the reducing agent, the metal of higher potential, is an insoluble agent serving as a specific means of recovering the copper from solution.

This review of the prior art shows that the technology for pretreating fluids such as biological fluids for subsequent redox-based analysis does not utilize insoluble oxidizing agents which insoluble oxidizing agents are subsequently removed by means based on their insolubility so as to produce a fluid suitable for redox-based analysis; there is no disclosure either of etas used in conjunction with such systems.

SUMMARY OF THE INVENTION

This invention relates to a system including kits, for the inactivation or the rendering non-interfering of interfering redox-active substances in a fluid by the use of redox-active compounds, a method for using the redox-active insoluble compounds to render inert the interfering substances in the fluid and the purified fluid free of the interfering compounds.

The system of the invention provides for highly improved analytical results such as would be obtained in the absence of the contaminant under comparable conditions.

The invention relates to disposable, non-machine or instrumentation-independent disposable devices, and to machine or instrumentation dependent devices, which are not customarily discarded after each use.

The invention disclosed overcomes the difficulties presented by reducing agents contaminating a fluid. The

invention involves a process for providing an amount of an oxidizing agent in excess of that required to render the contaminants non-interfering, of energy level enabling the spontaneous reaction with the contaminant reducing agent. The fluid also provides optionally an electron transfer agent to catalyze passage of electrons from the reducing agent to the added oxidizing agent. The oxidizing agent has the distinctive property to be insoluble in the fluid, so that it can be simply removed from the treated fluid by rapid, uncomplicated means such as filtration, decantation, sedimentation or centrifugation or other methods known in the art. The fluid treated in this manner is then prepared or ready by subsequent analysis or other use free from interference by either the contaminating reducing substances or residual oxidizing agent.

This invention is particularly useful in the pretreatment of certain biological fluids including, but not limited to, blood serum or plasma, cerebrospinal fluid, semen, saliva, tears, sinovial fluid, lymph or urine since these contain not only many important analytes, but also contain unknown and variable amounts of contaminating reducing agents. The invention is also useful for veterinary applications in the analysis of the biological fluids of animals.

The invention may also be applied to complex fluids containing contaminating oxidizing agents, wherein these contaminants would be destroyed by the addition of electron transfer agent and an insoluble reducing agent, that would be removed on the basis of its insolubility prior to use or analysis of the fluid. Thus the invention is applicable to a system and method of rendering non-interfering either oxidizing contaminants or reducing contaminants by an insoluble oxidizing agent or reducing agent, respectively.

The fluid to be treated can be aqueous partially aqueous or a non-aqueous (or organic) fluid which contains an analyte to be assayed as well as redox-active contaminants. The invention is broadly applicable to fluids which are

single or multi-phase; multi-phase fluids may be considered emulsions or suspensions.

The system of the invention, provides for the interfering-substance containing fluid to be contacted with the redox-active agent which is insoluble in the fluid. The interfering substance can be a reducing or an oxidizing compound. The insoluble redox-active compound undergoes a redox reaction with the interfering substance. Insoluble redox agent remaining in the fluid is then selectively removed from the fluid. The resulting fluid free of the interfering substance can then be analyzed for a particular analyte by oxidation-reduction analysis methods without the interfering redox contaminant that would cause inaccurate results. Thus, the analysis performed on the purified fluid is more reliable and accurate. Similarly any other use made of the fluid can be without the distorting or interfering effect that the redox-active contaminants would cause.

In addition to the use in machine-independent devices, the invention is applicable for use in machine-dependent diagnostic systems and methods whereby more accurate analysis of a fluid's analyte or analytes is possible. Generally the machine-independent devices are discarded after each use, the machine-dependent are not.

A particularly important embodiment of the invention is kits. The kits include a system for bringing the analyte containing fluid into contact with the RAIC so that the RAIC renders non-interfering substantially all the redox-active contaminants in the fluid. The kit provides a means for the selective passage of the treated fluid into a separate compartment or vessel without any residual RAIC. The kit provides compounds for an oxidation-reduction based analysis to take place in the compartment. This analysis is performed on the fluid which has had any redox-active contaminants as well as residual RAIC removed. Thus an accurate redox-based analysis for a particular analyte in the fluid can then be performed.

The invention also provides a method by oxidation for the removal, inactivation or the rendering non-interfering of interfering, redox-active contaminants which are contained in fluids. The invention also encompasses fluids such as biological fluids free of contaminants that would interfere in a redox reaction and render a redox-based analysis inaccurate. The invention encompasses biological fluids which are suitable for redox analysis of the fluid's redox-active analytes.

The system (including kits) and method of the invention also optionally contemplates the use of active electron transfer agents to facilitate the oxidation-reduction reaction between the insoluble redox-active agent and the redox-active interfering contaminant. The use of the electron transfer agent or agents in conjunction with the insoluble redox-active purifying compounds more effectively subject the interfering compound to a redox reaction.

Any residual redox-active insoluble agent which remains after the fluid has been treated, is removed by any means convenient to one skilled in the art using the insolubility property of the compound. Typical means include but are not limited to filtration, sedimentation, centrifugation or decantation. The fluid that remains is free of any residual redox-active agent as well as the redox-active contaminant material.

In one embodiment of the invention, the fluid to be purified is pretreated with the insoluble redox-active agent. The redox-active agent reacts with the redox-active contaminants contained in the fluid. After the fluid is recovered with the redox contaminants inactivated, removed or rendered non-interfering and any residual insoluble redox agent removed, the fluid is assayed for the particular analyte sought to be determined in a redox measuring system. These redox based measuring systems for analyzing a fluid include but are not limited to those described earlier in this application.

In another embodiment of the invention, the fluid to be purified and then analyzed passes into two reaction chambers. The first chamber (or area) contains the insoluble redox agent and may or may not contain an electron transfer agent. The fluid containing the contaminants passes through the first chamber where the redox contaminants are rendered non-interfering and then into a second chamber without carry over of the insoluble redox agent, where the fluid is assayed for particular analytes. These analytes are detected and concentration determined by a redox measuring system for analyzing a fluid including but not limited to those described earlier in this application. This embodiment permits the fluid or analyte to be treated and measured in one closed system, in one procedure and without having to recover the purified fluid from one system and transferring it to another system.

The insoluble redox-active agents selectively react with the redox-active contaminant when the analyte and the insoluble redox-active agent are present in the same fluid; they do not affect the fluid's analyte which is subsequently to be analyzed. Thus, the insoluble redox agent can be considered substantially inert with respect to the fluid's analyte to be analyzed.

Generally, the amount of insoluble redox-active agent used is that amount sufficient to eliminate the adverse effect of any interfering redox-active contaminant in the fluid to be analyzed.

Any excess insoluble redox-active agent is then removed as described elsewhere in this application.

Commonly the amount of insoluble redox-active agent used to treat one ml of bodily fluid ranges from about 1 to about 100 mg, preferably from about 5 to about 20mg. Preferably the insoluble redox-active agent is placed in a physical state that is most effective to render the fluid's contaminants non-interfering. It is desirable to increase as much as possible its reactive surface areas. For instance,

such a physical state includes that of a finely ground material.

Some of the more preferred insoluble redox-active agents are: lead (IV) dioxide (PbO_2), cuprous sulfide (Cu_2S), ceric dioxide (CeO_2), silver sulfide (Ag_2S), silver ferrocyanide (Ag_4 $Fe(CN)_6$), silver oxide (Ag_2O), manganese dioxide (MnO_2), barium permanganate ($BaMnO_4$), cuprous carbonate, potassium dichromate ($K_2Cr_2O_7$), potassium permanganate ($KMnO_4$), cuprous ferrocyanide, lead permanganate and the peroxyacetic acid derivative of carboxymethyl cellulose. The particular insoluble redox-active agent selected will depend on the fluid and analyte to be analyzed.

The redox-active agents in the invention belong to classes of compounds that can be identified as follows:

Various electron transfer agents specified hereinafter can be used to facilitate the reaction of RAIC and contaminants. However, the use of electron transfer agents is not necessary for this invention and is an optional ingredient.

These electron transfer agents are generally used in an amount in the range of about 0.1-10mM. The preferred range of amount of electron transfer agent is about 0.5mM-2mM. These electron transfer agents do not interfere with the subsequent redox analysis of the fluid.

It should be noted that this invention has special application where there is a need for an accurate analysis of a biological fluid. In particular blood, plasma or serum is often analyzed for particular analytes of interest such as cholesterol, blood urea nitrogen, ketones, glucose, lactate or triglycerides. Interfering contaminants with which this invention deals include, without limitation the following classes of compounds: redox active acids, thiols and bilirubin.

In blood these may include organic acids like ascorbic acid, as well as other substances such as uric acid, gentisic acid, bilirubin, glutathione, cysteine, and thiol-containing peptides and proteins such as albumins. The

specific nature of many contaminants that interfere in the redox-based analysis of a fluid are generally unknown. Various proteins themselves may be associated with contaminants which may be substituents thereon. In those circumstances, these are oxidized. The assaying or analyzing of other bodily fluid for glucose, or monosaccharides is also of interest for patients with diabetes. Interfering contaminants in such bodily fluids include uric acid, ascorbic acid and thiol-containing proteins and peptides. Nevertheless this invention has broader application than the analysis of biological fluids. Thus, it is apparent from the description provided that it is an object of the invention to render non-interfering those redox-active contaminants contained in the fluid to be analyzed that interfere with the detection and determination of the concentration of an analyte that is to be analyzed using enzymatic or oxidation-reduction chemistry.

Similarly it is an object of the invention to render non-interfering those redox-active contaminants in a fluid that interfere in the use of the fluid and to provide a fluid free of interfering redox-active contaminants so that said fluid will not be affected by the interfering substances.

Thus it is an object of the invention to provide a system and method of selectively reacting with the contaminant and which does not destroy, alter or inactivate the analyte and which does not affect the analyte to be analyzed or assayed by a redox-based reaction.

It is an object of the invention to provide a system and method for removing or inactivating from a fluid redox-active substances without leaving a new redox-active contaminant or a residue of insoluble redox-active agent after the fluid has been treated for the removal of the interfering contaminants.

It is also an object of the invention to provide a system and method which removes, inactivates, or renders non-interfering those interfering contaminants contained in a

fluid, when said fluid is to be analyzed by oxidation-reduction means as well as a means for assaying the analyte within one closed system without the need for removal and transfer of the fluid after the fluid is purified to another separate system.

It is also an object of the invention to provide disposable diagnostic test kits or apparatus-independent kits for accurately determining the presence or concentration of an analyte in a fluid by pretreating the fluid with the insoluble redox-active compound and analyzing the pretreated fluid by redox-based methods all within one kit.

It is also an object of the invention to pretreat biological fluids to remove contaminants so the pretreated fluid can be analyzed for particular analytes or used for other purposes free from interfering or distorting redox-active agents.

The invention is not limited to the objects listed above and these objects as well as other uses of the invention will become apparent to those skilled in the art and by the description which follows.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The invention provides a system of treatment or method performed on a fluid which can be aqueous, non-aqueous, or a mixture. The fluid generally contains a redox-active analyte or analytes which fluid contains a redox-active interfering contaminant. These contaminants are interfering in the sense defined earlier in that they interfere in the accurate analysis of a redox-active analyte in the fluid which is sought to be analyzed by a redox measurement system. The contaminants are also interfering in the sense that they cause error or non-ideal performance in the use of the fluid.

The invention provides a system and method whereby the fluid is treated with an insoluble redox-active agent. It is important to note that regardless of whether the fluid is aqueous, non-aqueous or partially aqueous, the redox-active agent used in this invention should be insoluble in

the fluid. The amount of insoluble redox-active agent introduced will depend on the fluid itself and the redox-active component to be inactivated by the redox reaction. The amount of insoluble redox-active agent used is that amount which is sufficient to render interfering redox-active contaminants non-interfering. In general an amount of insoluble redox-active agent is added which is in excess of that needed to insure the complete oxidation-reduction reaction of the insoluble redox-active agent with the redox-active contaminants.

The temperature at which the reaction is carried out is generally ambient temperature such as in the range of about 10°C to 50°C.

The reaction between the insoluble redox-active agent or agents or the redox-active contaminant or contaminants can be facilitated by the introduction of one or more electron transfer agents to the fluid.

After the fluid has been treated so that all or substantially all the interfering redox-active contaminants have undergone a redox reaction or have been rendered non-interfering, any remaining insoluble redox-active agent is removed. This removal is accomplished by means based on the insolubility of the redox-active agent.

The resulting fluid is free of redox-active contaminants as well as residual redox-active agent. This fluid is ideal for accurate analysis for redox-active analyte by redox-reaction measurement systems. The invention provides a fluid which is free in general of any residual non-specific reducing or oxidizing agent which would constitute an interference for the subsequent use of the fluid.

Thus the kit, system and method disclosed herein generally involves a set of two sequential redox reactions. The first reaction involves the RAIC and interfering redox-active contaminant. The second reaction is generally a NAD-NADH dependent redox reaction or a non-NAD-NADH oxido-reductase (enzymatic) redox reaction coupled to color

changing reactions which involves the determination of the concentration or presence of an analyte. A large number of color changing reactions such as the peroxidase-chromogen reactions for hydrogen peroxide can be coupled to these redox reactions.

The kit includes both those compounds necessary for rendering the contaminants non-interfering, and those compounds for a redox-based analysis. The kit typically includes a physical support or housing for two layers of filters between which is contained the RAIC. The fluid to be treated is generally passed through the first filter which may contain an electron transfer agent. The fluid dissolves the electron transfer agent, contacts the RAIC and passes through the second filter with the RAIC retained.

This invention is useful for removing interfering reducing substances from a liquid medium. This invention is particularly useful if the fluid to be treated is a sample that is to be analyzed by redox chemistry, or a sample where residual non-specific reducing or oxidizing agent constitutes an interference for the subsequent use of the fluid. The invention is especially useful if the fluid is a bodily fluid such as blood serum or plasma, amniotic fluid, cerebrospinal fluid, sinovial fluid, urine, saliva, tears, semen and the like, and the subsequent use is the analysis of the fluid for the concentration of a component of the fluid by enzymatic and other redox reactions.

As discussed above, the basic principles of redox chemistry and its use in measurement systems are well known. When an analyte is redox-active, it can be used in redox reactions to detect its presence and determine its concentration. Determination of an analyte by redox reactions, however, opens the possibility of interference by any non-analyte redox-active contaminant in the sample. Therefore, it is useful to have a means by which interfering redox-active substances can be eliminated without affecting the analytes that will subsequently be assayed by a redox

reaction, and without leaving a new redox-active contaminant in the fluid, such as residual oxidizing agent.

To accomplish the removal of interfering reducing agents, the use of insoluble oxidizing agents was contemplated. In this invention, insoluble means the inability to dissolve to any appreciable extent in the fluid of interest. As a general characteristic, appreciable solubility refers to the residual concentration, if any, of the insoluble agent relative to the analyte of interest, and in typical situations, the residual will be less than 1/1000 of the analyte concentration, and in any case, this residual concentration will typically be less than about 10 ppm in the product fluid. The oxidizing agent used in this invention is insoluble in the fluid to be treated or analyzed.

Obviously, the solubility of an oxidizing agent, as for any chemical composition, will depend upon the nature of the fluid. Manganese dioxide, for example, is insoluble in water but soluble in 11N HCl. Potassium dichromate is soluble in water but insoluble in alcohols. Solubilities of organic and inorganic oxidizing (and reducing) agents in a wide variety of solvents are well known to those skilled in the art, and are extensively compiled, for instance, in the Tables of Physical Constant of Inorganic and of Organic Compounds, found in the Handbook of Chemistry and Physics, CRC Publications, Cleveland, OH. Also for the purpose of this invention, oxidizing agents will mean any chemical composition having an electromotive potential such that it would react with another chemical composition, called the reducing agent, having different electromotive potential, where electrons are transferred spontaneously from the reducing agent to the oxidizing agent if a means or pathway for the electron transfer was available. In accordance with the invention an oxidizing compound may be used with an electromotive potential such that it can react with an interfering reducing contaminant.

Numerous treaties describing the very wide scope of redox-active chemical compositions have been published and

are available in chemical references, as is well known to those of average skill in the art. The extensive compilations of the standard reduction potentials of inorganic and organic compounds may be consulted to readily determine the relative reducing or oxidizing strength of any particular inorganic or organic compound. The compounds characterized by a highly positive standard reduction potential are preferred reducing agents. The actual occurrence of a redox reaction depends upon the combined potentials of the oxidizing and reducing agents under a particular set of conditions, including the availability of a pathway or means for the electrons to transfer between the two agents. In particular, the standard reduction potentials refer to redox-active species at 1 Molar concentration in solution.

Thus from among the published compendia, it would not involve undue experimentation to select compounds having both a relatively potent oxidizing (or reducing) activity, and insolubility in any particular solvent. The means to make such selections are quite well known to anyone of average skill in the art.

While the selection of insoluble oxidizing (and reducing) agents can be so made it is not obvious that insoluble oxidizing (or reducing) agents would actually effectively react with other redox partners. This may be because the insignificant solution concentration of insoluble agents would abolish the inherent redox capability or that the reaction would be prevented by the necessity for electron transfer across a physical phase barrier, or for other specific reasons.

In fact, attempts to destroy the contaminating reducing activity of human plasma by addition of various insoluble oxidizing agents were only partly successful. When, for example, plasma was contacted with crystals of manganese (IV) dioxide, there occurs some diminution of the plasma capacity to reduce indicators like ferricyanide ion, but sufficient reducing activity remains to consume several

tenths of mM of this agent; and, for example, to reduce the tetrazolium prodye MTT to give a colored formazan. This reducing capacity of plasma did not change even after hours of agitated contact of the plasma with the insoluble oxidizing agents.

An analysis of the contaminating reducing activity remaining after MnO_2 treatment demonstrated that all of the remaining activity was located in the trichloracetic acid (TCA) precipitated fraction. The reducing contaminants in the TCA soluble fraction were destroyed by manganese dioxide treatment. It is well known that TCA will only precipitate macromolecular polymers, e.g., proteins. Therefore, apparently manganese dioxide is incapable of reacting with protein associated reducing contaminants, either because it is not of sufficient oxidizing strength, or because no pathway exists for transfer of electrons between the polymeric contaminant and the insoluble manganese dioxide.

It was therefore surprising, when treatment of the plasma-MnO_2 suspension by very small amounts of a wide variety of electron transfer agents, overcame this poor reaction between the contaminating reducing substances and the insoluble oxidizing agent. Plasma treated with 1 mM electron transfer agent and contacted for a few seconds with solid, insoluble oxidizing agent, completely lost the ability to reduce ferricyanide, or to reduce tetrazolium salts to the formazan. Moreover, pretreatment of plasma in this manner did not affect the concentration of any of the common components of blood, serum of plasma usually assayed by redox chemistry, except those components that are highly redox-active and directly able to reduce oxidizing agents like ferricyanide. Thus uric acid, glutathione and ascorbic acid, for example were destroyed, but cholesterol, triglycerides, amino acids (except cysteine), and so forth were unaffected.

There are occurrences where complete destruction of all reducing contaminants in plasma would occur in the absence of an electron transfer agent. These occurrences are advantageous in situations where, for example, the electron

transfer agent would interfere with subsequent analysis. We discovered that lead (IV) dioxide insoluble oxidation agent is capable of destruction of all the contaminating reducing activity in plasma. An electron transferring substance is not needed for the action of PbO_2, and both the TCA soluble and insoluble contaminants are destroyed. It is surprising that PbO_2 should be an acceptable agent. The standard oxidation potential of lead is 1.455 volts, considerably greater than the 1.229 volt reduction potential of oxygen. Thus, lead (IV) dioxide has the energetic capacity to split water, suggesting that it might not be stable in an aqueous solution. Stability is observed, however, probably due to the extreme insolubility of the lead oxidizing agent.

Thus in accordance with the invention the removal of contaminating reducing (or oxidizing) agent was accomplished by the pretreatment of a fluid with an insoluble oxidizing (or reducing) agent, especially when the fluid was also treated with a soluble electron transfer agent to provide more effective pathways for electrons to the insoluble agent. The resultant fluid is free of both oxidizing and reducing activity and will be especially suitable for uses where such residual activity constitutes an interference (but where the presence of the electron transfer agent does not constitute such interference). Of special utility would be fluids to be subsequently analyzed by methods involving the unmasking of specific reducing (or oxidizing) activity by specific redox catalysts such as redox-active enzymes. Of course, the invention is not intended to be limited to analytical uses of fluids pretreated in accordance with the invention, and indeed, the general spirit and teaching of this invention makes obvious a multitude of other uses to those of average skill in the art.

In accordance with the invention, the system comprises the treatment of some fluid, that fluid either hydrophilic (aqueous solutions), hydrophobic (lipid-like), or any mixture of the two. An insoluble oxidizing agent is introduced into the fluid so that the agent will contact the

interfering contaminants. Some of the more preferred insoluble redox-active agents are: cuprous sulfide (Cu_2S), ceric dioxide (CeO_2), silver sulfide (Ag_2S), silver ferrocyanide ($Ag_4\ Fe(CN)_6$), silver oxide (Ag_2O), manganese dioxide (MnO_2), barium permanganate ($BaMnO_4$), potassium dichromate ($K_2Cr_2O_7$), potassium permanganate ($KMnO_4$), cuprous ferrocyanide, lead permanganate and the peroxyacetic acid derivative of carboxymethyl cellulose. The use of a particular insoluble redox-active agent will depend on the fluid and analyte to be analyzed.

Generally the amount of insoluble oxidizing agent to be used is that amount sufficient to render non-interfering any interfering reducing agents in the fluid to be analyzed, but is usually an amount in excess of that amount needed to insure the complete oxidation of all contaminants. This amount is a function of the surface area the insoluble redox-active agent provides for effective contact with the interfering contaminant in the fluid. Since the redox-active agent is insoluble it can only react with the interfering contaminant at its surface area in contact with the interfering contaminant. Thus a smaller quantity of an insoluble redox-active agent with a large surface area such as a finely ground powder would render contaminants non-interfering just as effectively as a larger quantity of the insoluble redox-active agent such as one lumped mass.

It is also be noted that the liquid medium itself and the contaminant to be rendered non-interfering also affect the amount of insoluble redox-active agent to be used. The reaction of the insoluble oxidizing (or reducing) agent may be further facilitated by inclusion of small amounts, usually in the range of about 0.1-2 mM of any of a variety of electron transfer agents, not limited to but including the group consisting of phenazine methosulfate, 2,6-dichlorophenolindophenol, substituted 1,4-benzoquinones including 1,4-benzoquinone, mono-, di-, tri- and tetra-substituted 1,4-benzoquinones where the substituents may include, but are not limited to, simple alkyl groups

containing 1 to 5 carbons, halogens, alkoxyl radicals with 1 to 4 carbons, among other possible substitutions.

Substituted 1,4-benzoquinones of particular utility in this regard are 2,5- and 2,6-dimethyl-1,4-benzoquinone, 2,5- and 2,6-dichloro-1,4-benzoquinone, 2-isopropyl-5-methyl-3,6-dibromo-1,4-benzoquinone, 2-methoxy-5-methyl-1,4-benzoquinone, and 2,3-dimethoxy-5-methyl-1,4-benzoquinone. Substituted and unsubstituted 1,4-naphthoquinones, and 1,2-benzoquinones are also generally useful as electron transfer agents in this invention. A large collection of redox-active dyes generally members of the phenazine, phenoxazine, phenothiazine and indamin families of dyes also function well as electron transfer agents, although they are usually not preferred because they impart a color to the final treated fluid. An extensive compilation of dyes in these families, all of which function to some degree as electron transfer agents in this invention, is given in "H.J. Conn's Biological Stains 9th Ed," R.D. Lillie, Ed, Williams and Wilkins, Baltimore, MD (1977).

Treatment methods in accordance with the invention can be any method which will accomplish the objectives described herein. In one method analyte is passed over a bed of the insoluble oxidizing (or reducing) agent. The bed may contain an amount of oxidizing agent so that all contaminating reducing agents are oxidized as the analyte passes over the bed. The surface area of the bed is preferably sufficiently large so that all analyte comes in contact with oxidizing agent as the analyte flows through the bed. The bed of oxidizing agent may be supported on a porous filter matrix that retains the oxidizing agent while allowing the fluid to pass through rapidly.

In another embodiment of the invention, the insoluble oxidizing agent is sandwiched between two membranes. The analyte is introduced through the top of one membrane, flows through the layer of oxidizing agent, then passes through the second membrane. The membrane allows the rapid passage of fluid, but retain the oxidizing agent so

that it is separated from the fluid. The kit represents a specific physical disposable embodiments of the system.

In a further embodiment of the invention, the oxidizing agent is bound to a substrate. The substrate is insoluble, and can consist of, but is not limited to membranes, polymers, and columns of some supporting material. Binding the oxidizing agent to an insoluble support has the effect of rendering the oxidizing agent insoluble. Analyte is then passed through the oxidizing agent which is attached to the support substrate. In this embodiment, filtration is not required since the analyte will not carry any of the bound oxidizing agent with it.

In a further embodiment of the invention, insoluble oxidizing agent and analyte can be mixed together in some container, then mixed for an amount of time sufficient to allow all analyte to be contacted by the oxidizing agent. The oxidizing agent can then be removed from the analyte by filtration, decantation, sedimentation or centrifugation.

In each of the embodiments mentioned above, to the fluid there may be added about 0.1-2 mM of an electron transfer agent prior to contacting the insoluble oxidizing agent. In embodiments involving the sandwiching of oxidizing agent between membrane or filter supports, or involving oxidizing agent attached to an insoluble substrate, the electron transfer agent may be introduced to the fluid dried on a support layer that constitutes the first layer of the sandwich, such that the fluid contacts this layer just prior to contacting the oxidizing agent. In such an embodiment, some of the electron transfer agent leaches into the fluid in passage to the oxidizing agent. The embodiment involving a support layer with electron transfer agent dried on, followed by a sandwich of oxidizing agent may, in certain configurations, permit the fluid to be actively driven through the structure holding the sandwich layers. This embodiment is particularly efficient and rapid for pretreating the fluid.

In the practice of this invention, the amount of insoluble oxidizing agent used is set by the dimensions of the support system. The support system is designed in such a way to offer enough surface area of insoluble oxidizing agent so that all of the analyte flowing through the system will be contacted by the oxidizing agent. Optionally, if the surface area of the oxidizing agent is not large, as in the case where oxidizing agent and analyte are mixed together in a container, then the length of contacting time has to be sufficiently long to allow the oxidation of all contaminating reducing agents.

In the preferred embodiment of the invention, there are two reaction chambers into which the analyte is made to pass. A chamber in this case consists of any enclosed are designed to contain the fluid being analyzed, and can be open at one or both ends and be of any desired shape and size. The first chamber contains the insoluble oxidizing agent, and is designed for the analyte to be introduced into one end of the chamber, then pass through to the other end readily, where it enters the second chamber. This second chamber contains the redox measuring system, and can include but is not limited to one of the redox measuring systems described in the Background and Prior Art Section of this patent application. The first chamber or pretreatment chamber, contains enough insoluble oxidizing agent so that all contaminating reducing substances are rapidly reduced as the fluid passes through. If the oxidizing agent used consists of loose, insoluble particles, there is provided a filter between the two chamber. If the oxidizing agent is bound to an insoluble support, such filter is unnecessary. It is an advantage of this invention that the analyte does not have to first be treated separately by one procedure wholly apart from the redox measuring system, then introduced into the measuring system is a separate step - the analyte is treated then passes directly to the measuring system. It is also an advantage that treatment is rapid, and does not interfere

with obtaining accurate results from the redox measuring system.

The filters, filter matrices, and membranes which have been discussed in connection with embodiments of the invention are insoluble, hydrophilic, synthetic or natural polymers.

Examples of this invention are presented below. It is not intended for these examples to limit the invention. Furthermore, it is not intended that this invention be limited only to the pretreatment of fluids that will subsequently be analyzed. This invention is applicable in all cases where fluids contain redox-active components that can be eliminated by the use of redox-active insoluble compounds.

The following examples illustrate the system and method of the invention and said example are offered by way of illustration and not by way of limitation.

Example 1

A solution of 100 mg/dL glucose was prepared, and dosed with 1 mM dithiothreitol (DTT). This solution was then measured with a glucose enzyme electrode. An erroneous result was obtained based on the known amount of glucose put in the solution.

Two ml of the solution was run through a bed of insoluble oxidizing agent. This bed was created by laying down 200 mg of cuprous sulfide (Cu_2S) evenly over a 1 cm diameter piece of filter paper, seated in a cylinder over a receiving vessel. The glucose solution was then measured again using the same enzyme electrode, and the result was correct, based on the concentration of glucose placed in the solution. Therefore, DTT was acting as a contaminating reducing agent.

Example 2

A solution of sarcosine and the chromogen dimethyl thiazolyl diphenyltetrazoliumbromide (MTT) was made up. When sarcosine dehydrogenase is added, sarcosine is oxidized and MTT is reduced, yielding formaldehyde, glycine and colored

MTT-formazan. Sarcosine concentration can be measured colorimetrically, since MTT-formazan will absorb at 580 nm. A reaction was run, with conditions as follows:

50 uM sarcosine

1 IU/mL sarcosine dehydrogenase

0.5 mM MTT

20 mM potassium phosphate; pH 7.5

The reaction was run to endpoint, then absorbance was read on a spectrophotometer at 580 nm, and found to be 0.48.

The reaction was repeated, but 0.5 mM sodium ascorbate was introduced. This time, color production was so intense that the absorbance was off scale. Ascorbate is an interfering reducing substance, and will readily reduce MTT.

A 1 ml solution of sarcosine containing ascorbate was placed in a sealed 1.2 ml microcentrifuge tube with 50 mg of cerium oxide (CeO_2), then inverted gently for 5 minutes. This treated fluid was then run in the above colorimetric redox measuring system again, and the absorbance obtained was 0.49, very close to the expected value. Therefore, ascorbate was oxidized but sarcosine was not.

Example 3

The test in example 2 was repeated, but CeO_2 was replaced with silver sulfide (Ag_2S). Results were similar to those obtained in example 2. Before treatment with Ag_2S, color production was off scale due to the presence of ascorbate, but after treatment, the expected amount of color production occurred.

Other insoluble silver (I) compounds, such as silver ferrocyanide ($Ag_4\ Fe(CN)_6$) and silver oxide (Ag_20), were also found to perform similarly.

Example 4

In a reaction that contains:

100 mM Tris acetate pH 9.5

0.5 mM MTT

2 mM NAD

2 mM ferricyanide
5 IU/mL diaphorase
2 IU/mL lactic acid dehydrogenase

a dark purple color is generated if greater than 1 mM lactic acid is added, but the reaction will remain light yellow when less than 1 mM lactic acid is added. This is because ferricyanide is preferentially reduced by NADH before the formazan, and 1 mM is the threshold concentration.

This set of conditions was run with 0.2 mM uric acid. Concentrations of only 0.9 mM or greater of lactic acid were required to get purple color. Uric acid is a contaminating reducing agent; in the example, it reduced ferricyanide, thereby lowering the threshold response. The uric acid-containing analyte fluid was run through a filter apparatus that was overcoated with finely ground MnO_2, then run again in this colorimetric redox measuring system. This time, the threshold color response was found to be at the expected value. Therefore, uric acid was oxidized by the MnO_2, but lactic acid was not.

Example 5

The conditions in example 4 were repeated, but the MnO_2 was replaced with insoluble permanganate salts, such as barium permanganate ($BaMnO_4$). Before treatment with these oxidizing agents, the threshold response was lowered - color production occurred with lower concentrations of lactic acid than expected based on the ferricyanide concentration. After treatment, the threshold response was back up to the expected level. $BaMnO_4$ was effective in oxidizing the uric acid, but did not affect the lactic acid.

Example 6

In a particular reducing sugar assay, monosaccharides are determined by oxidation in an alkaline solution by potassium ferricyanide, and the reduction of ferricyanide is measured by the decrease in absorbance at 420 nm. This is a redox measurement system that does not employ a protein catalyst.

When a 50 mg/dL solution of D-mannose was spiked with uric acid, then run in the reducing sugar assay with ferricyanide, the decrease in A420 was greater than expected. An insoluble oxidizing filter was formed by making a slurry of MnO_2 and cellulose fibers, then drying down to form the filter. The mannose/uric acid solution was passed through this filter at neutral pH. The solution was made basic by the addition of sodium hydroxide, since mannose is not reducing unless the pH is high, then run in the reducing sugar assay again, this time with the expected results. Therefore, uric acid was oxidized but mannose was not.

Example 7

Some oxidizing agents are soluble in aqueous solutions, but they are not soluble in an organic solvent, that is, non-aqueous fluid. This is true for potassium dichromate ($K_2Cr_2O_7$), which is soluble in water, but not in ethanol.

When a solution of formaldehyde in ethanol is diluted into a solution containing sodium carbonate and silver nitrate, a black color forms, indicating the presence and oxidation of the formaldehyde. The same result will be obtained if mercaptoethanol is diluted into the carbonate/ silver nitrate solution. Therefore, if formaldehyde and mercaptoethanol are in solution together, this redox measurement system cannot distinguish between the two. If formaldehyde is the compound being detected, mercaptoethanol can be considered a contaminating reducing compound.

A 1 mL solution of 5 mM formaldehyde was made in absolute ethanol with 1 mM mercaptoethanol. To remove the mercaptoethanol, this solution was run through a 1 cm diameter piece of filter paper on top of which had been spread 100 mg $K_2Cr_2O_7$, and collected into a test tube. An aqueous solution containing 0.28 M sodium carbonate and 12 mM silver nitrate was added to an aliquot of the filtrate, whereupon a black color formed, indicating the presence of formaldehyde. The filtrate was further analyzed by the addition of carbonate-buffered Ellman's reagent. This would

cause a bright yellow color to be generated if any mercaptoethanol had passed through the dichromate filter. No yellow color occurred, indicating that the mercaptoethanol had been oxidized by the dichromate filter. When potassium permanganate replaced the dichromate, essentially the same results were obtained.

Example 8

Human plasma was analyzed for its lactate concentration by incubating an aliquot of freshly prepared plasma in a reaction mixture containing:

3 mM NAD
8 U/ml lactate dehydrogenase
40 mM HEPES buffer, pH 8.5
50 % plasma
5 U/ml Clostridial diaphorase
2.00 mM potassium ferricyanide

Lactate was oxidized by the lactate dehydrogenase with concomitant reduction of NAD to NADH. In the presence of diaphorase and ferricyanide, the NADH was reoxidized with concomitant reduction of 2 mols/mol of ferricyanide. Ferricyanide reduction was directly determined by absorbance at 420 nm in a spectrophotometer. For a particular plasma sample, the apparent total lactate content was 1.9 mM. When this plasma was incubated without the lactate dehydrogenase under the conditions above, 0.50 mM ferricyanide was reduced directly, with an additional 1.40 mM ferricyanide being reduced when the lactate dehydrogenase was added. Thus, the plasma contained an interfering reducing activity equivalent to 0.5 mM lactate in the native plasma.

When this plasma was pretreated by amendment with 1 mM 2,5-dichloro-1,4-benzoquinone and addition of 100 mg/ml CeO_2, followed by filtration of the insoluble oxidizing agent, the assay now showed no measureable reduction of the ferricyanide in the absence of the lactate dehydrogenase and a value corresponding to 1.4 mM lactate in the plasma.

Example 9

Measurement of plasma cholesterol in a machine-independent, disposable device. 50 ul of human plasma were placed in a plastic tube containing a sandwich of filter papers. Suitable filter papers were prepared from cellulose, nitrocellulose, nylon, fiberglass or polycarbonate polymers. The first filter paper contained 1 umol of 2-isopropyl-5-methyl-3,6-dibromo-1,4-benzoquinone. Beneath this paper a bed 1 mm thick of finely ground MnO_2 was contained, held in place a bed 1 mm thick of finely ground MnO_2 was contained, held in place by a second filter paper. The end of this tube was constricted, mechanically holding the sandwich in place, with an opening going to a 20 ul capillary containing a dried film that, when rehydrated by entrance of aqueous fluid, would give a solution of 0.1 M potassium phosphate, pH 7.4, 2% sodium cholate, 200 U/ml porcine pancreatic cholesterol esterase, 20 U/ml cholesterol oxidase, 1 mM thiazolylblue tetrazolium (MTT), 3% trans-1,2-cyclooctanediol, and 12.4 mM potassium ferricyanide. A firmly fitting plunger is used to drive the plasma through the sandwich and into the capillary. In the capillary, the plasma hydrates the reagents, causing hydrolysis of cholesterol esters by the cholesterol ester hydrolase, and oxidation of the cholesterol by the oxidase. In the presence of the quinone electron transfer agent and the exclusion of oxygen by the walls of the capillary, the electrons made available by the oxidation reduce the ferricyanide. When all the ferricyanide is reduced, electrons then and only then reduce the tetrazolium dye giving a sharp color change by formation of the highly colored fomazan dye. The concentration of cholesterol in a particular plasma was 230 mg/dl, equal to 5.94 mM. This did not exceed the equivalent ferricyanide concentration (12.4/2 = 6.20 mM) so this plasma caused reduction of 11.9 mM ferricyanide, leaving 0.5 mM ferricyanide intact and causing no reduction of MTT and no formation of the darkly colored dye.

When the insoluble oxidizing agent was removed from the sandwich, and the test repeated in an otherwise identical device, a dark color formed, incorrectly indicating that the cholesterol content exceeded 240 mg/dl, the concentration at which the ferricyanide is just consumed and color changes begin. This falsely high reading was due to unknown reducing substances in the plasma that lowered the ferricyanide concentration, allowing cholesterol oxidation to reduce the tetrazolium. In the system containing both electron transfer agent and insoluble oxidizing agent, these unknown reducing substances were advantageously removed, permitting the chemical color switch to correctly and precisely indicate the presence of less than 240 mg/dl cholesterol. In other similarly constructed devices, the manganese dioxide was replaced with the insoluble oxidizing agents cuprous ferrocyanide, or lead permanganate, with essentially equivalent results.

Example 10

Measurement of hydrogen peroxide. A solution of 0.05 M Tris chloride, pH 7.7 contained 2.4 mM hydrogen peroxide and 0.4 mM ascorbic acid. The hydrogen peroxide content of the solution was measured by dilution of 1 volume into 19 volumes of a solution containing 20 U/ml horseradish peroxidase, 0.1 M potassium phosphate pH 7.3, 0.8 mM 4-aminoantipyrine and 20 mM 4-hydroxybenzoic acid. The solution was allowed to react for 10 min, and the absorbance was read at 500 nm in a spectrophotometer. From a standard curve produced with standard solutions of hydrogen peroxide, the color of the test solution indicated that the peroxide concentration of the original solution was 2.0 mM, 0.4 mM less than the true concentration. Passage of the test solution over a bed of the insoluble oxidizing agent barium permanganate prior to assay as above, resulted in a color corresponding with the true concentration. Thus, the ascorbic acid contaminating the solution interfered with the peroxide-dependent oxidation of the prodye components, giving a falsely low color intensity, and the insoluble oxidizing

agent quickly and completely removed this interfering reducing agent without affecting the peroxide concentration. When similar measurements were carried out, but the hydrogen peroxide solution was contaminated with cysteine, or uric acid, or L-dopa, similar results were obtained, that is, without the pretreatment the essentially true concentration of peroxide was not indicated by the color reaction, but the essentially true value was obtained if the fluid was pretreated with the insoluble oxidizing agent.

Example 11

Measurement of glucose in bodily fluids. A 20-ul aliquot of human blood was diluted to 1 ml in a reaction mixture containing 15 U/ml D-glucose oxidase from Aspergillus niger and a hydrogen peroxide measuring mixture consisting of 1 mM of 0.1 M sodium phosphate, pH 7.0; 20 mM 3,3',5,5'-tetramethylbenzidine and 25 U/ml horseradish peroxidase. The color at 660 nm was determined in a spectrophotometer after 10 min reaction at 37°C and the glucose concentration obtained with reference to a standard curve, the glucose content was found to be 4.85 mM. When the same blood sample was assayed by measurement of oxygen consumption in an oxygen electrode, correcting for any oxygen consumed before addition of the enzyme, the glucose content was found to be 5.25 mM. When this blood sample was filtered through a bed of glass fibers to remove red blood cells, and then passed through a sandwich containing 5 umols 2,6-dimethyl-1,4-benzoquinone and a 1 mm deep bed of MnO_2, the resultant plasma measured 5.23 mM glucose in the glucose oxidase, peroxidase/benzidine color reaction described above. Thus, the interfering reducing substances present in the blood were removed from the plasma by the insoluble oxidizing agent and electron transfer agent prior to analysis, permitting an essentially correct measurement of the glucose content.

When this analysis was repeated, but using a sample of neonatal cerebrospinal fluid, the glucose content measured without pretreatment by insoluble oxidizing agent was 1.82 mM glucose. After pretreatment the glucose content was found to

be 2.45 mM and in the oxygen electrode, the content was determined to be 2.45 mM. As in well known in the art, cerebrospinal fluid is subject to greater relative error in glucose determination than is blood due to the typically lower glucose concentrations and the typically higher content of reducing substances like ascorbic acid.

Example 12

Measurement of cholesterol in plasma. A sample of human plasma was diluted 20-fold into a measurement reaction containing 0.1 M Tris chloride pH 7.5, 20 U/ml pancreatic cholesterol esterase, 20 mM sodium cholate, 0.2% Triton X-100, 5 U/ml Nocardia cholesterol oxidase, 0.4 mM 4-aminoantipyrine, 10 mM phenol and 25 U/ml horseradish peroxidase. The mixture was incubated for 20 min at 37°C and the color determined at 500 nm in the ususal manner. By comparison to a standard curve prepared with authentic cholesterol standards dissolved in a mixture of 5% 2-propanol and 1% Brij 35, the color of the test mixture was related to cholesterol concentration. The cholesterol content was found to be 4.87 mM (equivalent to 188 mg/dl). However when this plasma was reacted with cholesterol oxidase in an oxygen electrode, in the presence of the reagents specified above but without the antipyrine, phenol and peroxidase, oxygen consumption corresponded to a cholesterol content of 5.23 mM (202 mg/dl). If the plasma was pretreated by amendment with 0.5 mM of 2,5-dichloro-
1-4-benzoquinone and passage through a bed of finely ground cerium dioxide, and then assayed as before in the colorimetric reaction mixture, the measured cholesterol content was found to be 5.20 mM, in excellent agreement with the value determined by the electrode. Thus the pretreatment with insoluble oxidizing agent and electron transfer agent removed unidentified reducing substances in the plasma that were responsible for the falsely low measurement.

Example 13

Interfering reducing substances were rendered non-interfering by an organic oxidizing agent attached to an

insoluble matrix. The peroxyacetic acid derivative of carboxymethyl cellulose (CMC) was prepared by incubating 100 g CMC with 100 ml 90% hydrogen peroxide for 6 h at room temperature, and the derivatized product washed extensively with water to remove the hydrogen peroxide. The wet peroxyacetic derivative was mixed with an equal weight of cellulose carrier and the mixture dried to 50% relative humidity at 20°C. Twenty mg of this matrix was placed in the interior of a glass tube and used for the pretreatment of 100 ul of the plasma described in example 12, by forcing the plasma through the tube with a plunger. The cholesterol content of the plasma following this treatment was measured by the esterase/oxidase/peroxidase color system described in example 12 to be 5.24 mM, in excellent agreement with the correct value. Thus, this insoluble organic oxidizing agent also destroyed the contaminating reducing substances in the plasma, without affecting the analyte concentration.

Example 14

Coating Glass Fiber Filter Material with Lead (IV) Dioxide. A suspension of 5 grams of lead (IV) dioxide in 100 ml of a 2% methylcellulose (15 cp) aqueous solution was spread over 600 square centimeters of glass fiber filter material having a mean pore size of 3.0 microns (S&S 31 glass fibers available from Schleicher & Schuell). The coated filter was washed free of methylcellulose with water and dried at 100°C. Prior to use, small circles of the material were punched or cut out of this material. Trated filter material could be made in advance and stored for later usage.

Example 15

Pretreatment of Plasma by Lead (IV) Dioxide Prior to Cholesterol Measurement. A small circle of glass fiber filter material precoated with lead (IV) dioxide, as discussed above, was inserted into the bottom of a 1 ml disposable plastic syringe. A sample of plasma, of approximately 0.5 ml was added to the syringe, and the plasma pushed through the filter at such a rate so that at least 1 sec of filter time occurred. The cholesterol content of the

treated plasma was subsequently measured in a Ciba Corning Impact 400E clinical analyzer, using the manufacturer's cholesterol reagent and run accoring to the manufacturer's specifications.

IN THE CLAIMS:

1. A diagnostic instrumentation-independent kit disposable after use which is adapted to accurately determine by means of an oxidation-reduction reaction, the presence (or absence) and concentration of an organic analyte in a biological fluid which contains redox-active contaminants which are likely to interfere with the analytical redox determination of the analyte, which kit comprises a fluid insoluble oxidizing compound and separate therefrom, the reactants for a redox reaction which measure the analyte in the organic fluid, wherein the contaminants are devoid of adverse effect on the redox reaction, which redox reaction determines more accurately the concentration of the analyte than would be determined in the absence of the insoluble oxidizing compound.

2. The kit of claim 1 wherein the insoluble oxidizing compound is positioned in a first area and the reactants for a redox reaction are positioned in a second area.

3. The kit of claim 2 wherein the first and second areas are separated by an insoluble hydrophilic natural or synthetic polymer, which is impermeable to the insoluble oxidizing compound.

4. The kit of claim 2 wherein the contaminants in the fluid have been reacted with the insoluble oxidizing compound in the first area thereby causing the contaminants in the fluid to be oxidized and non-interfering in the redox reaction in the second area.

5. The kit of claim 3 wherein the insoluble oxidizing compound is selected from the group consisting of lead (IV) dioxide, manganese dioxide, cuprous ferrocyanide, lead permanganate, cuprous carbonate, cuprous sulfide, ceric dioxide, silver sulfide, silver ferrocyanide, silver oxide, barium permanganate, potassium dichromate, potassium permanganate or a peroxyacetic acid derivative of carboxymethyl cellulose.

6. The kit of claim 2 wherein the insoluble oxidizing compound is insoluble in the fluid which contains the analyte.

7. The kit of claim 6 wherein the insoluble oxidizing compound is water insoluble.

8. The kit of claim 6 wherein the insoluble oxidizing compound is organic solvent insoluble.

9. The kit of claim 6 wherein the insoluble oxidizing compound is an oxidizing compound which changes the contaminants to the oxidized state.

10. The kit of claim 6 wherein the insoluble oxidizing compound is supported by a porous polymeric matrix which is permeable to the fluid containing the analyte.

11. The kit of claim 2 which also contains an electron transfer agent positioned in the first area wherein the insoluble oxidizing compound is positioned, which electron transfer agent promotes the redox reaction of the insoluble oxidizing compound with the contaminants.

12. The kit of claim 2 wherein the biological fluid is selected from the group consisting of blood, plasma, serum, amniotic fluid, cerebrospinal fluid, sinovial fluid, saliva, urine, semen and tears.

13. The kit of claim 12 wherein the contaminant substance of the biological fluid is selected from the group consisting of cysteine, ascorbic acid, mercaptoethanol, uric acid, dithiothreitol, methyldopa, gentisic acid, dipyrone, ampyrone, homogentisic acid, glutathione, thiol-containing peptides and proteins, and thiols.

14. A system for purifying a biological fluid of contaminant redox-active reducing substances which are likely to adversely interfere with an analytical redox determination of an analyte in the biological fluid.

which system comprises the biological fluid containing the contaminant reducing substances and an insoluble oxidizing compound in the fluid which renders by redox reaction the contaminant substances non-interfering in the analytical redox determination of the analyte in the

fluid, whereby after reaction of the insoluble oxidizing compound with the contaminant substance, an improved analytical redox determination of increased accuracy of the analyte in the biological fluid can be carried out without adverse effect due to the contaminants.

15. The system of claim 14 which includes means for removing from the fluid the insoluble oxidizing compound after the interfering contaminants have been rendered non-interfering.

16. The system of claim 15 wherein the removing means is a means adapted to remove the insoluble oxidizing compound using the insolubility property of said insoluble oxidizing compound.

17. The system of claim 15 wherein the removing means is an insoluble support substrate.

18. A system for purifying a biological fluid of contaminant redox-active reducing substances which are likely to adversely interfere with an analytical redox determination of an analyte in the biological fluid.

which system comprises a first area which contains the biological fluid and an insoluble oxidizing compound in the fluid which is capable of rendering by redox reaction the contaminant substances non-interfering with the analytical redox determination of the analyte in the fluid, a second area which comprises the reactant for a redox-reaction of the analyte in the biological fluid to determine the presence (or absence) or concentration of the analyte in the biological fluid, and means for separating the first and second area, which means is permeable to the analyte in the fluid but not to the insoluble oxidizing compound.

19. The system of claim 18 which includes also in the first area an electron transfer agent which promotes the redox reaction of the insoluble oxidizing compound with the contaminants.

20. The system of claim 18 wherein the biological fluid in the first area also contains the contaminant reducing substances.

21. The system of claim 18 wherein the contaminants in the fluid have been reacted with the insoluble oxidizing compound in the first area thereby causing the contaminants in the fluid to be oxidized and non-interfering in the redox reaction in the second area.

22. The system of claim 18 wherein the separating means also holds the insoluble oxidizing compound.

23. The system of claim 14 wherein the insoluble oxidizing compound is selected from the group consisting of manganese dioxide, cuprous ferrocyanide, lead permanganate, potassium permanganate, cuprous sulfide, ceric dioxide, silver sulfide, silver ferrocyanide, silver oxide, barium permanganate, potassium dichromate, cuprous carbonate or a peroxyacetic acid derivative of carboxymethyl cellulose.

24. The system of claim 18 wherein the insoluble oxidizing compound is selected from the group consisting of lead (IV) dioxide, manganese dioxide, cuprous ferrocyanide, lead permanganate, potassium permanganate, cuprous sulfide, ceric dioxide, silver sulfide, silver ferrocyanide, silver oxide, barium permanganate, potassium dichromate, cuprous carbonate, or a peroxyacetic acid derivative of carboxymethyl cellulose.

25. The system of claim 14 wherein the biological fluid is selected from the group consisting of blood, plasma, serum, amniotic fluid, cerebrospinal fluid, sinovial fluid, saliva, semen, urine and tears.

26. The system of claim 18 wherein the biological fluid is selected from the group consisting of blood, plasma, serum, amniotic fluid, cerebrospinal fluid, sinovial fluid, saliva, semen, urine and tears.

27. The system of claim 14 wherein the contaminant substances are selected from the group consisting of cysteine, ascorbic acid, mercaptoethanol, uric acid, dithiothreitol, methyldopa, gentisic acid, dipyrone, ampyrone, homogentisic acid, glutathione, thiol-containing peptides and proteins and thiols.

28. The system of claim 18 wherein the contaminant substances are selected from the group consisting of cysteine, ascorbic acid, mercaptoethanol, uric acid, dithiothreitol, methyldopa, gentisic acid, dipyrone, ampyrone, homogentisic acid, glutathione, thiol-containing peptides and proteins and thiols.

29. A method for the treatment of an organic fluid and for measuring with improved accuracy the presence (or absence) or concentration of an organic analyte in the organic fluid which contains redox-active contaminants which are likely to adversely interfere with an analytical redox determination of the analyte by means of an oxidation-reduction reaction which comprises:

adding to the fluid which contains said contaminants an insoluble oxidizing compound,

reacting said contaminants and the insoluble oxidizing compound, thereby causing the contaminants not to adversely interfere in the oxidation-reduction reaction,

removing the insoluble oxidizing compound from the fluid,

mixing with the fluid which is free of the insoluble oxidizing compound, reactants for an oxidation-reaction of the analyte, and

measuring the presence (or absence) or concentration of the analyte in the fluid, which measurement of the analyte is more accurate because the reduced contaminants do not adversely affect said measurement.

30. The method of claim 29 wherein the fluid which contains said contaminants and the insoluble oxidizing compound are located in one area and the reactants for the oxidation-reduction reaction for determination of the analyte in the fluid are located in a second area, the first and second areas being separated by separating means permeable to the passage of the analyte in the fluid but not to the insoluble oxidizing compound.

31. The method of claim 30 which comprises passing the fluid from the first area to the second area through the

separating means thereby also causing the passage of the contaminants which are now devoid of adverse effect on the redox determination into the second area.

32. The method of claim 31 wherein the fluid in the first area also includes an electron transfer agent which promotes the redox reaction of the insoluble oxidizing compound with the contaminants.

33. The method of claim 31 which comprises also removing the insoluble oxidizing compound after contacting the contaminants by means which uses the insolubility property of said compound in the fluid.

34. The method of claim 33 wherein the removing is performed by precipitation, decantation, sedimentation, filtration or centrifugation.

35. The method of claim 31 wherein the contaminants in the fluid have been reacted with the insoluble oxidizing compound in the first area thereby causing the contaminants in the fluid to be oxidized and non-interfering in the redox reaction in the second area.

36. The method of claim 30 wherein the fluid is selected from the group consisting of blood, plasma, serum, amniotic fluid, cerebrospinal fluid, sinovial fluid, saliva, urine, semen and tears.

37. The method of claim 30 wherein the insoluble oxidizing compound is selected from the group consisting of lead (IV) dioxide, manganese dioxide, cuprous ferrocyanide, lead permanganate, potassium permanganate, cuprous sulfide, ceric dioxide, silver sulfide, silver ferrocyanide, silver oxide, barium permanganate, potassium dichromate, cuprous carbonate, or a peroxyacetic acid derivative of carboxymethyl cellulose.

38. The method of claim 30 wherein the contaminant substances of the biological fluid are selected from the group consisting of cysteine, ascorbic acid, mercaptoethanol, uric acid, dithiothreitol, methyldopa, gentisic acid, dipyrone, ampyrone, homogentisic acid, glutathione, thiol-containing peptides and proteins and thiols.

39. A biological fluid which contains an organic analyte the presence (or absence) or concentration of which is to be measured in an organic fluid with increased accuracy which fluid comprises

reactants for a redox reaction to determine the presence (or absence) or concentration of the analyte, and

redox-active interfering contaminants that have been rendered non-interfering by redox reaction with an insoluble oxidizing compound, which insoluble oxidizing compound has been removed from the fluid.

40. The biological fluid of claim 39 which is selected from the group consisting of blood, plasma, serum, amniotic fluid, cerebrospinal fluid, sinovial fluid, saliva, urine, semen and tears.

41. The biological fluid of claim 39 in which the interfering contaminant is selected from the group consisting of cysteine, ascorbic acid, mercaptoethanol, uric acid, dithiothreitol, methyldopa, gentisic acid, dipyrone, ampyrone, homogentisic acid, glutathione, thiol-containing peptides and proteins and thiols.

42. The biological fluid of claim 39 in which the insoluble oxidizing compound is selected from the group consisting of lead (IV) dioxide manganese dioxide, cuprous ferrocyanide, lead permanganate, cuprous sulfide, ceric dioxide, silver sulfide, potassium permanganate, silver ferrocyanide, silver oxide, barium permanganate, potassium dichromate, cuprous carbonate, or a peroxyacetic acid derivative of carboxymethyl cellulose.

43. The kit of claim 1 wherein the fluid insoluble oxidizing compound is lead (IV) dioxide.

44. The system of claim 14 wherein the insoluble oxidizing compound is lead (IV) dioxide.

45. The system of claim 18 wherein the insoluble oxidizing compound is lead (IV) dioxide.

46. The method of claim 29 wherein the insoluble oxidizing compound is lead (IV) dioxide.

47. The fluid of claim 39 wherein the insoluble oxidizing compound is lead (IV) dioxide.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/01887

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): C12Q 1/26, 1/32; G01N 1/00
U.S. CL.: 435/25, 26; 436/175

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.	435/25, 26, 28, 14, 176, 177, 805 436/175, 177, 178

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

Chemical Abstract Services Online (file CA, 1967-1990);
Automated Patent System (File USPAT, 1975-1990_ See Attached Search Terms

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X Y	US, A, 4,281,062 (KALLIS) 28 July 1981, See column 2, lines 53-68; and column 5, lines 1-62	1-10, 12-18, 33-42 11, 19, 32, 43-47
X Y	US, A, 4,298,688 (KALLIES) 03 November 1981, See column 3, lines 11-23; column 5, lines 53-68; column 6, lines 1024	1-10, 12-18 33-42 11, 19, 32, 43-47
Y	US, A, 4,310,626 (BURKHARDT ET AL) 12 January 1982, See the abstract; column 3, lines 49-62; and column 4, lines 23-33.	1-47
A,P	US, A, 4,897,346 (GAWRONSKI) 30 January 1990, See the abstract; column 4, lines 1-37; and column 5, lines 29-65.	1-47

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [3]	Date of Mailing of this International Search Report [3]
05 JULY 1990	16 AUG 1990
International Searching Authority [1]	Signature of Authorized Officer [20]
ISA/US	TONI R. SCHEINER

Form PCT/ISA/210 (second sheet) (May 1986)

ATTACHMENT TO FORM PCT/ISA/210, PART II.

II. FIELDS SEARCHED/SEARCH TERMS:

interference ?

interfering

interfere #

cysteine

ascorb ?

permanganate #